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Abstract: Cardiac hypertrophy is an adaptive response that normalizes wall stress and compensates for increased workload. It is accompanied by distinct qualitative and quantitative changes in the expression of protein isoforms concerning contractility, intracellular Ca2+-homeostasis and metabolism. Changes in the myosin subunit isoform expression improves contractility by an increase in force generation at a given Ca2+-concentration (increased Ca2+-sensitivity) and by improving the economy of the chemo-mechanical transduction process per amount of utilised ATP (increased duty ratio). In the human atrium this is achieved by partial replacement of the endogenous fast myosin by the ventricular slow-type heavy and light chains. In the hypertrophic human ventricle the slow-type -myosin heavy chains remain unchanged, but the ectopic expression of the atrial myosin essential light chain (ALC1) partially replaces the endogenous ventricular isoform (VLC1). The ventricular contractile apparatus with myosin containing ALC1 is characterised by faster cross-bridge kinetics, a higher Ca2+-sensitivity of force generation and an increased duty ratio. The mechanism for cross-bridge modulation relies on the extended Ala-Pro-rich N-terminus of the essential light chains of which the first eleven residues interact with the C-terminus of actin. A change in charge in this region between ALC1 and VLC1 explains their functional difference. The intracellular Ca2+-handling may be impaired in heart failure, resulting in either higher or lower cytosolic Ca2+-levels. Thus the state of the cardiomyocyte determines whether this hypertrophic adaptation remains beneficial or becomes detrimental during failure. Also discussed are the effects on contractility of long-term changes in isoform expression of other sarcomeric proteins. Positive and negative modulation of contractility by short-term phosphorylation reactions at multiple sites in the myosin regulatory light chain, troponin-I, troponin-T, -tropomyosin and myosin binding protein-C are considered in detail

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Review

Modulation of contractility in human cardiac hypertrophy by myosin essential light chain isoforms

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Abstract

Cardiac hypertrophy is an adaptive response that normalizes wall stress and compensates for increased workload. It is accompanied by distinct qualitative and quantitative changes in the expression of protein isoforms concerning contractility, intracellular Ca^{2+}-homeostasis and metabolism. Changes in the myosin subunit isoform expression improves contractility by an increase in force generation at a given Ca^{2+}-concentration (increased Ca^{2+}-sensitivity) and by improving the economy of the chemo-mechanical transduction process per amount of utilised ATP (increased duty ratio). In the human atrium this is achieved by partial replacement of the endogenous fast myosin by the ventricular slow-type heavy and light chains. In the hypertrophic human ventricle the slow-type β-myosin heavy chains remain unchanged, but the ectopic expression of the atrial myosin essential light chain (ALC1) partially replaces the endogenous ventricular isoform (VLC1). The ventricular contractile apparatus with myosin containing ALC1 is characterised by faster cross-bridge kinetics, a higher Ca^{2+}-sensitivity of force generation and an increased duty ratio. The mechanism for cross-bridge modulation relies on the extended Ala–Pro-rich N-terminus of the essential light chains of which the first eleven residues interact with the C-terminus of actin. A change in charge in this region between ALC1 and VLC1 explains their functional difference. The intracellular Ca^{2+}-handling may be impaired in heart failure, resulting in either higher or lower cytosolic Ca^{2+}-levels. Thus the state of the cardiomyocyte determines whether this hypertrophic adaptation remains beneficial or becomes detrimental during failure. Also discussed are the effects on contractility of long-term changes in isoform expression of other sarcomeric proteins. Positive and negative modulation of contractility by short-term phosphorylation reactions at multiple sites in the myosin regulatory light chain, troponin-I, troponin-T, α-tropomyosin and myosin binding protein-C are considered in detail.

Keywords: Cardiac hypertrophy; Heart failure; Cross-bridge kinetics; Contractility; Myosin heavy chain; Myosin light chain; Ca^{2+}-sensitivity–tension relation

1. Introduction

Heart muscle performs work with high contractile efficiency [1,2]. Under increased workload it reacts with hypertrophy. This represents an adaptive response in order to normalise wall stress and compensate for the increased hemodynamic load [3]. When the load is chronically elevated, compensated hypertrophy may progress to pump failure. Except in the case of acute global ischemia which is accompanied by a collapse of energy production, it is not clear which process, or processes, are responsible for turning compensated hypertrophy into heart failure. Numerous physiological characteristics concerning metabolism, contractility and cellular structure are found to be impaired in heart failure. However, contractile function is often preserved or even improved in hypertrophy as well as in failure when studied at the subcellular level, i.e. on permeabilised muscle fibers or on isolated proteins in the in vitro motility assay and in solution studies.

The changes in the contractile apparatus (Figs. 1 and 2) result from long-lasting changes in the gene expression program (reviewed in [4,5]). Because cardiomyocytes are

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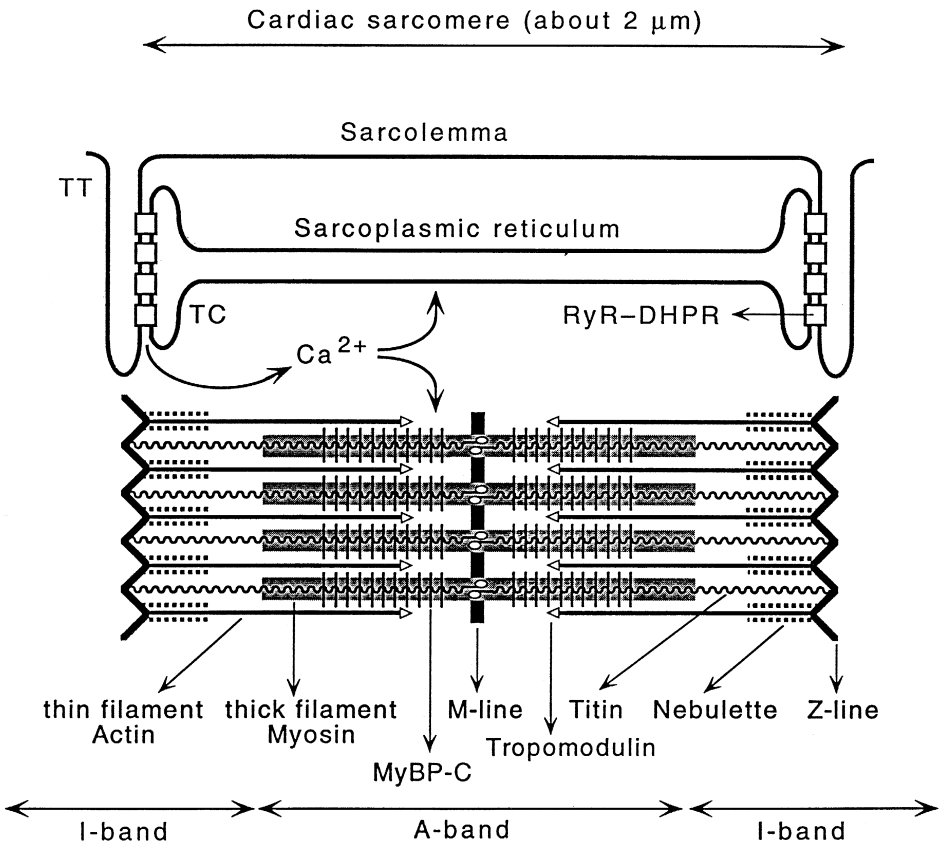

Fig. 1. Scheme of the cardiac sarcomere in relation to the membranous structures responsible for the intracellular Ca^{2+}-handling. TT, transverse tubule; TC, terminal cisternae; DHPR, dihydropyridine receptor (voltage sensor and slow inward Ca^{2+}-channel) at the TT membrane; RyR, ryanodine receptor (Ca^{2+}-release channel of the SR) with foot structures at the TC membrane [183]. M-line consists of myomesin, 165 kDa M-line protein and creatine kinase; Z-line contains mainly α-actinin, desmin and CapZ protein [32]. MyPB-C localizes to the eleven transverse stripes on either side of the M-line and binds to myosin and titin. Titin is anchored with its N-terminus at the Z-line and, running along the myosin filament, reaches the M-line with its C-terminal head portion where it interacts with myomesin and the M-line protein. Two nebulette molecules are associated with each actin filament, which originate with their C-terminus from the Z-line, and Tropomodulin caps the actin filaments at their pointed end [5].

no longer able to increase muscle mass by proliferation they resort to hypertrophy. Hypertrophy does not mean more of the same but involves specific qualitative alterations in the cell phenotype [5–7]. Reexpression of 'fetal genes' which are normally active in the fetal period coding for proteins such as β-myosin heavy chain (β-MHC) (see Table 1 for abbreviations), α-skeletal and α-smooth muscle actin or atrial natriuretic factor, may occur. The development of hypertrophy in humans is a slow and chronic process extending over years. However, the long-term changes in expression of contractile proteins is not simply a reactivation of the 'fetal gene program', but follows a pattern that seems to be determined by the physiological demands. These physiological demands are transmitted by a panoply of different stimuli, besides mechanical loading, involving hormones, catecholamines, growth factors, cytokines and vasoactive peptides (reviewed in [8]). Most of these hypertrophic stimuli use different intracellular signaling pathways which results in individual phenotypic responses as characterised by gene expression pattern, cell morphology and function. The effects of various hypertrophic stimuli are likely to be balanced in vivo, even during compensatory hypertrophy. However, the disturbance of any single factor may tip this balance for the worse. The precise knowledge of the function of the contractile apparatus including its regulatory mechanisms in the normal heart and during hypertrophy is indispensable when considering potential therapeutic strategies aimed at avoiding development of heart failure.

The purpose of this review is to put together recently published results on the structure and function of the myosin light chain (MLC) isoforms whose expression changes during the development of cardiac hypertrophy. The discussion includes the position and relation of the MLC in the myosin head next to the motor domain, the variation in MHC and MLC isoforms and their possible hybrid molecular assemblies in atrial and ventricular muscles. The knowledge how calcium controls the myosin cross-bridge kinetics is instrumental in delineating the details of the mechanism by which the atrial MLC1 (ALC1) positively affects contractility of the ventricular muscle. The pathophysiological consequences of the expression of

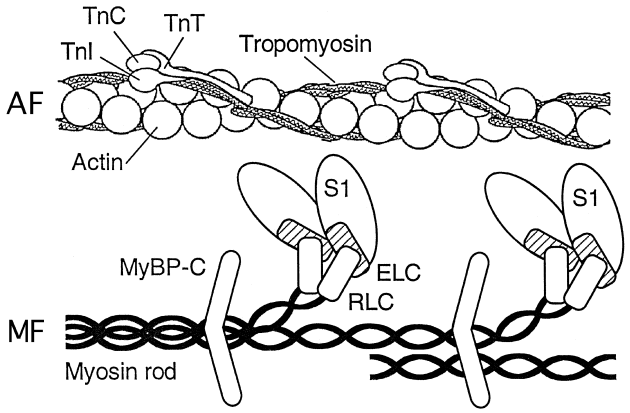

Fig. 2. Schematic detail of the sarcomere with the proteins affecting contractility and which are described in the text. AF, thin actin filament; MF, thick myosin filament; inhibitory (TnI), Ca^{2+}-binding (TnC) and tropomyosin binding (TnT) troponin components; S1, myosin subfragment-1 (myosin head portion); ELC and RLC, essential and regulatory myosin light chains; MyBP-C, hypothetical localization of the myosin binding protein-C spaced 43 nm apart along the myosin filament. The M-line would be to the left and the Z-line to the right.

the ALC1 are discussed in view of the functional viability of the cardiomyocyte with regard to intracellular Ca^{2+}-handling and energy supply during hypertrophy and in heart failure. In order to recognize the relevance of modulation of contractility by myosin essential light chain, we give an overview of other sarcomeric proteins associated with the contractile machinery and point out the role of protein phosphorylation in Ca^{2+}-sensitivity and cross-bridge kinetics. Taken together, we provide evidence for the functional significance of the change in MLC isoform expression in the human heart ventricle where the MHC remain unaltered during hypertrophy. In view of space limitation, reviews which contain references to primary information are often cited.

2. The myosin motor

The myosin muscle motor (myosin type-II) is a hexameric protein (520 kDa) consisting of two heavy chains (MHC) of around 220 kDa (1930–1940 amino acid residues) and of two pairs of light chains (MLC) of around 20 kDa each. The MHC subunits, which provide both the motor and the filament forming properties, can each be divided into two functional domains: the globular N-terminal head domain (corresponding to the proteolytic subfragment-1 or S1 of 120 kDa) and the elongated α-helical domain that, together with the corresponding domain of the second MHC, forms the coiled coil C-terminal rod (Fig. 2). The S1 has an approximate length of 16.5 nm and the rod domain one of 140 nm. The S1 domain can be further subdivided into two parts, (i) the motor domain, running from the N-terminus through to residue 770 and containing the nucleotide binding pocket and the actin binding cleft, and (ii) the 8 nm long α-helical light chain binding or regulatory domain (neck region) which extends from residue 771 to residue 843 and links the motor domain to the rod domain [9,10] (Fig. 3). The α-helix of this regulatory domain is stabilized by non-covalent association of one MLC of each type. Near its C-terminus the α-helix bends sharply at the residues W829–P830–W831 and this last stretch of the α-helix points along the myosin filament axis towards the middle of the sarcomere, before it joins the second MHC in the rod. The precise interactions of the MLC with the MHC in this region have been revealed by crystal structures of the chicken S1 [9] and of the regulatory domain of the molluscan scallop myosin [11]. The two MLC are located in series and are wrapped around the MHC in an anti-parallel orientation. In the chicken S1, MLC type-1 (essential light chain) binds to residues from L783 to M806 and MLC type-2 (regulatory light chain) to residues from E808 to L842 of MHC [9]. It

Table 1
Abbreviations, names and explanations

A1, atrial (αα)-MHC myosin	pCa, negative decadic logarithm of the free Ca^{2+}-concentration
A2, atrial (ββ)-MHC myosin	PKA, protein kinase-A
ALC1, atrial myosin essential light chain	PKC, protein kinase-C
ALC2, atrial myosin regulatory light chain	RLC, myosin regulatory light chain
CAMK, Ca^{2+}-calmodulin dependent protein kinase	S1, myosin subfragment-1 (myosin head portion)
cAMP, cyclic adenosine monophosphate	SDS-PAGE, sodium dodecylsulfate polyacrylamide gel electrophoresis
DCM, dilated cardiomyopathy	SERCA2, cardiac Ca^{2+}-pump of the SR
DTNB, dithiobis-nitrobenzoic acid	SR, sarcoplasmic reticulum
EF, ejection fraction	Tm, tropomyosin
EF-hand, Ca^{2+}-binding helix–loop–helix motif	Tn, troponin complex
ELC, myosin essential light chain	TnC, Ca^{2+}-binding Tn component
FHC, familial hypertrophic cardiomyopathy	TnI, inhibitory Tn component
ICM, ischemic cardiomyopathy	TnT, Tm-binding Tn component
MHC, myosin heavy chain	V1, ventricular (αα)-MHC myosin
MLC, myosin light chain	V2, ventricular (αβ)-MHC myosin
MLCK, myosin light chain kinase	V3, ventricular (ββ)-MHC myosin
MyBP-C, myosin-binding protein-C	VLC1, ventricular myosin essential light chain
NYHA, New York heart association classification	VLC2, ventricular myosin regulatory light chain

Single amino acid residues are given by the three-letter code and protein sequences in the one-letter code.

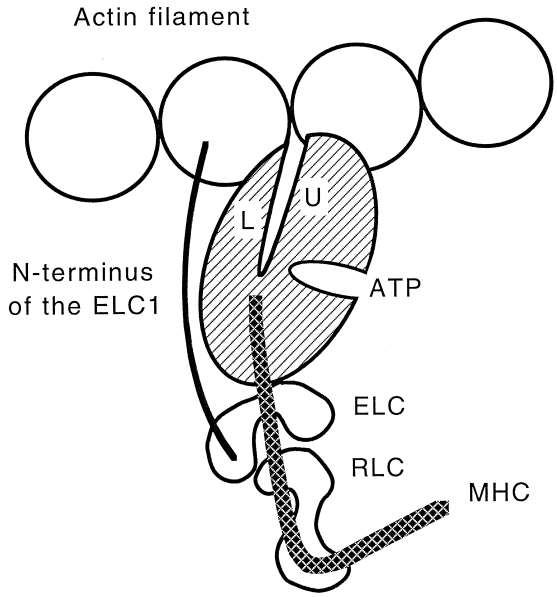

Fig. 3. Schematic drawing, approximately to scale, of the interactions of the myosin head (subfragment-1) with the actin filament. The myosin motor domain (hatched) displays the ATP binding pocket and the actin binding cleft. The upper (U) and lower (L) portions of the 50 kDa segment are interacting with two adjacent actin monomers. MHC denotes the α-helix which constitutes the regulatory domain with the two bound light chains (ELC and RLC). The extended N-terminus of the ELC1 reaches to the proximate actin. This state represents the rigor conformation as modeled from the crystal structure [23]. The Z-line would be to the left and the M-line to the right. For further details see text.

has been agreed to give the position numbers for amino acids in the sequence of the chicken skeletal muscle MHC even when referring to mammalian skeletal or heart muscle myosins [12,13].

Myosin cross-bridges (myosin head portions protruding from the myosin filament) provoke movement during contraction by repetitive interactions with the actin filament in the sarcomere. Thereby the cross-bridges are thought to swing through a number of power strokes, each time displacing the two filaments relative to one another by a distance of 5 to 10 nm [14,15]. Recent studies on time-resolved X-ray diffraction of live frog sartorius muscles [16] and fluorescence polarisation coupled with rapid length steps imposed on permeabilised rabbit psoas fibers (mechanically or chemically skinned fibers) [17], show attachment of cross-bridges with actin and synchronized head movements in the elementary force-generating process. However, the rowing cross-bridge model as accepted in the textbooks since 1971 [18] requires an update. The tip of the motor domain is now envisaged to bind actin with a more or less fixed geometry throughout the power stroke, while it is the distal C-terminal light chain binding region of the myosin molecule that actually moves [10,17,19]. The α-helix of this regulatory domain serves as a 'lever arm' to amplify relatively restricted rotational movements among different structural parts within the motor domain. It was shown that the length of the lever arm is directly proportional to the sliding velocity in an in vitro motility assay system [20]. For this purpose, the lever arm of the S1 domain of myosin-II from the slime mould Dictyostelium discoideum, a myosin that is homologous to vertebrate striated muscle myosin, was altered in length by addition or subtraction of light chain binding regions by genetic engineering. The advent of nano-technology has recently allowed to determine discrete stepwise movement averaging 11 nm under low load and single force transients averaging 3 to 4 pN under isometric conditions of rabbit fast skeletal muscle myosin [21]. In this case, myosin molecules were fixed on silica beads and one molecule was let to interact with an actin filament held at both ends by a feedback enhanced laser trap system.

X-ray structural data of the individual proteins together with data obtained by fiber diffraction and electron microscopy have been used to build detailed models of the rigor interaction of myosin with the actin filament [22–24]. In the contracting muscle this state is thought to occur transiently at the end of the power stroke after release of the products Pi and ADP before a new substrate ATP molecule gets bound to myosin. In the rigor state one myosin head is suggested to interact with two adjacent actin monomers (Fig. 3). A number of hydrophobic residues on the opposing faces of actin and myosin contribute to the main contacts. These contacts are flanked by charged myosin surface loops which form predominantly ionic interactions with adjacent regions of actin. Two such loops represent the connector regions between the segments of the MHC of S1 that can be obtained by proteolysis with trypsin, i.e. the 25-kDa, 50-kDa and 20-kDa segments starting from the N-terminus [25]. These loops exhibit considerable sequence variability and it appears that their composition influences the kinetic behavior of myosin from different sources. Replacement of the 50–20-loop in Dictyostelium myosin with the equivalent myosin sequence from rabbit fast skeletal, chicken smooth or rat cardiac muscle leads to an actin activated ATPase activity that corresponds to the one of the respective parent myosin [25]. The 25–50-loop has been suggested to affect the release of ADP from the nucleotide binding pocket and thus may be responsible for the observed differences in motility between different myosins [26]. In addition, and as discussed in the section on MLC below, the N-terminus of the large isoform of the essential MLC1 has also been shown to interact with the C-terminus of an actin monomer (Fig. 3) and to affect the actin activated ATPase activity.

3. Cardiac myosin heavy chains

At least eight different MHC types are expressed in mammalian striated muscles: two developmental isoforms (MHC-embryonic and MHC-perinatal), four fast isoforms (MHC-fast 2A, 2B, 2X/D and MHC-extraocular) as well

as MHC-slow which is identical to the cardiac β-MHC. In addition, the heart also expresses the cardiac α-MHC isoform (for review see [27]). The human α-MHC contains 1939 [28] and the β-MHC 1935 [29] amino acid residues. Among them they share 93.1% sequence identity. The rat α-MHC contains 1938 and the β-MHC 1935 residues [30], and they share 93.2% sequence identity. A total of 131 residues differ between them and most of these differences are confined to regions of biological significance in the S1 subfragment such as the N-terminus, the ATP binding pocket, the actin binding cleft, the light chain binding domain and in the two hinge regions further down in the rod domain. In inter-species comparison between human and rat, α-MHC shows over 97% sequence identity. The same holds for β-MHC.

During development and under pathophysiological conditions, the expression of the two cardiac MHC isoforms is regulated in a tissue-specific manner. Their expression is under additional hormonal control, notably by thyroid hormones [5,31,32]. In all mammalian species α-MHC is expressed in the atria throughout life. In small mammals such as rat and mouse, α-MHC is also the predominant isoform in the ventricles post partum and during adulthood. In addition, α-MHC is expressed in extraocular muscles as well as in some mandibular muscles of carnivores. β-MHC is expressed in the embryonic/fetal ventricles of all mammals and disappears soon after birth in small animal species. In larger animals such as rabbit, dog, pig and human, β-MHC remains the predominant ventricular isoform throughout adulthood (Table 2).

The fact that the β-MHC is also expressed in skeletal muscle fibers of the slow type-1 allows to perform functional studies on mutated β-MHC from patients with familial hypertrophic cardiomyopathy (FHC). In the in vitro motility assay of purified myosin from the soleus muscle of FHC patients with seven different point mutations in the β-MHC gene, the velocity of actin translocation was on average about half or less than that of myosin from healthy control persons (average control value 0.48 $\mu m\ s^{-1}$) [33]. In addition, myosin prepared from interventricular septal sites as well as from the soleus muscle of FHC patients with the R403Q (position 405 in chicken) mutation exhibited the same low motility (0.11 and 0.13 $\mu m\ s^{-1}$, respectively). Permeabilised slow fibers from the soleus muscle of FHC patients with either the R403Q or G741R (position 743 in chicken) mutations also showed impaired contractile functions, although the fraction of mutant MHC in these fibers was not known [34].

4. Cardiac myosin light chains

The myosin light chains (MLC) comprise two subfamilies, the essential light chains (ELC) and the regulatory light chains (RLC). Both MLC subfamilies belong, together with the calmodulin and the troponin-C subfamilies, to the superfamily of intracellular Ca^{2+}-binding proteins that characteristically contain four EF-hand domains (helix–loop–helix motif) [35]. However, during evolution, all four EF-domains of vertebrate muscle myosin ELC have lost their ability to bind Ca^{2+}, while in the case of the RLC only the first EF-domain can bind either Ca^{2+} or Mg^{2+} with high affinity in situ in the myosin molecule (binding affinities in the range of $10^7\ M^{-1}$) [36]. The ELC is called 'essential' because it was originally thought to be essential for the hydrolytic activity of myosin; but later, this proved not to be the case [37,38]. Alternative names are alkali MLC (since ELC can be removed from myosin by elevated pH) or MLC1. The RLC is termed 'regulatory' because in vertebrate smooth muscle and non-muscle cells, contractile activity is triggered by phosphorylation of this myosin light chain [39]. Alternative names are DTNB-MLC (since it can be removed from myosin by DTNB-treatment), PLC (it can be reversibly phosphorylated) or MLC2. The ATPase activity of isolated myosin does not seem to be affected by the ELC or RLC. It has however become increasingly evident, that within the complex sarcomeric structure, the MLC are involved in the fine-tuning of the contractile activity.

Table 2
Myosin heavy chain (MHC) and MLC1 isoform composition in all four chambers of six explanted hearts from patients with end-stage heart failure compared to five hearts from patients with compensated hemodynamics. Muscle specimens were obtained from the free wall of all four heart chambers. Relative content of MHC and MLC1 was densitometrically evaluated after electrophoretic resolution [68,69]. Type of disease and functional state of the patients is given in the text. Average percentages ± SEM are given

Heart condition	Protein isoform	Right atrium	Left atrium	Right ventricle	Left ventricle
Compensated hearts	α-MHC	90 ± 2.1	93 ± 3.4	< 3	< 3
	β-MHC	10 ± 2.1	7 ± 3.4	100	100
	ALC1	100	100	< 1	< 1
	VLC1	< 1	< 1	100	100
Failing hearts	α-MHC	52 ± 9	56 ±	< 3	< 3
	β-MHC	48 ± 9	44 ± 9	100	100
	ALC1	90.3 ± 2.1	91.9 ± 3.3	5.5 ± 1.4	7.8 ± 4.4
	VLC1	9.7 ± 2.1	8.1 ± 3.3	94.5 ± 1.4	92.2 ± 4.4

Two varieties of ELC and RLC are expressed in the heart, characteristic for the atrial and the ventricular tissues. They are thus designated ALC1 and VLC1 for atrial and ventricular ELC, respectively, and ALC2 and VLC2 for the corresponding RLC species. ALC1 and VLC1 (human and rat) contain 190–200 (Table 3) and the VLC2 around 165 amino acid residues (no data available for ALC2) [35]. The molecular mass for the MLC1 and MLC2 types is around 22 and 19 kDa, respectively. However, the MLC1 migrates with a higher apparent mass of around 27 kDa in SDS gel-electrophoresis because of its particular N-terminus. The most striking difference between the two types of MLC concerns an additional peptide stretch of around 30 residues at the N-terminus of the ELC. An unusual accumulation of around 10 Ala and 10 Pro is found in this extra stretch. In addition, several positively and negatively charged amino acid residues are clustered near the N-terminus (Table 3). Such sequences rich in Ala and Pro form rigid extended structures [40]. It has been shown by biochemical in solution studies that the N-terminus of fast skeletal muscle ELC (ELC1fast) binds to the C-terminus of actin [41,42]. This extended N-terminal structure of the ELC1fast cannot be seen in the crystal structure of the chicken S1 subfragment [9]. The last visible residue in the fast skeletal muscle ELC corresponds to positions 46–53 in the sequences of human and rat ALC1 and VLC1, and this residue lies roughly in the centre of the 3-dimensional structure of the ELC at a distance of 7–8 nm from the actin filament surface. Thus a stretch of around 50 residues is available for the N-terminus of ALC1 and VLC1 to bridge the gap of 7–8 nm and to contact the actin filament. In principle, only 24 residues are required to span a distance of 8 nm if present in an extended β-sheet conformation. Taken together, the odd 50 N-terminal residues in ALC1 and VLC1 are ample to run along the myosin head moiety and to interact with a proximate actin monomer (Fig. 3) [24]. It has recently been shown that the first 11 N-terminal residues of human ALC1 are responsible for binding to the actin C-terminus [43]. The interaction of the N-terminus of ELC has indeed been shown to affect contractility and actin activated ATPase activity in skeletal muscle protein systems (for reviews see [44–47]). It is this structural feature that seems to be of relevance for the pathophysiological aspects of cardiac hypertrophy discussed below. Interestingly, all ELC isoforms expressed in vertebrate striated muscles have such an extended N-terminus that is found neither in invertebrate ELC nor in the vertebrate smooth and non-muscle myosin ELC.

The ALC1 is identical to the embryonic ELC1emb which is also transiently expressed in fetal skeletal muscles as well as in fetal ventricular heart tissue [48]. On the other hand, the VLC1 is identical to the skeletal muscle ELC1bslow which is expressed in adult slow skeletal muscles [49]. VLC2 is also expressed in adult slow skeletal muscles as RLC2slow. An additional ventricular RLC designated VLC2 * that has a different isoelectric point but the same mass as VLC2 and can be resolved by 2-dimensional gel electrophoresis (Fig. 4), is found in ventricles of most mammals [50,51]. Both VLC2 varieties have different amino acid sequences and each contains one phosphorylation site for a Ca^{2+}-calmodulin dependent light chain kinase (MLCK) [52]. In contrast, the sole ALC2 variety in the human atrium can be phosphorylated at two sites. The significance of this feature for cardiac contractility is, however, not known [53].

Table 3
Amino acid sequence and charge distribution of the first 20 residues of the pre-domain of the myosin light chain type-1 (MLC1) from human and rat cardiac and fast skeletal muscles. Numbering is based on the mature protein, neglecting the N-terminal Met [47]. Following the pre-domain, the rest of the MLC1 comprises the four EF-domains with their respective interdomains and end with the last residue of the fourth EF-domain (this part contains 143 amino acid residues in each case). For comparison the charge distribution is also given for the last 20 residues in the sequence of mammalian cardiac α-actin. Bold characters represent residues with positive charge; outline characters represent residues with negative charge. Data taken from Refs. [35,184]

	Number of charges in first 20 residues of MLC1 or in last 20 resiues of actin	First 20 amino acid residues of myosin light chain pre-domain or last 20 residues in actin	Number of residues of entire MLC1 pre-domain	Total residues of MLC1 or of actin
		1 20		
Human ALC1	7	A P **K K** P 𝔼 P **K K** 𝔼 A A **K** P A P A P A P	– 53 –	196
Rat ALC1	7	P P **K K** P 𝔼 P **K K** 𝔼 T A **K** V A A A P A P	– 49 –	192
Human VLC1	9	A P **K K** P 𝔼 P **K K** 𝔻 𝔻 A **K** A A P **K** A A P	– 51 –	194
Rat VLC1	9	A P **K K** P 𝔼 **K K** 𝔻 𝔻 A **K** T A A P **K** A A P	– 55 –	198
Human ELC1fast	5	A P **K K** 𝔻 V **K K** P V A A A A A A P A P A	– 52 –	195
Rat ELC1fast	5	A P **K K** 𝔻 V **K K** P A A A A P A P A P A P	– 49 –	192
Cardiac α-actin	6	– W I S **K** Q 𝔻 Y 𝔻 𝔼 A G P S I V H **R K** C F		375
		366 375		

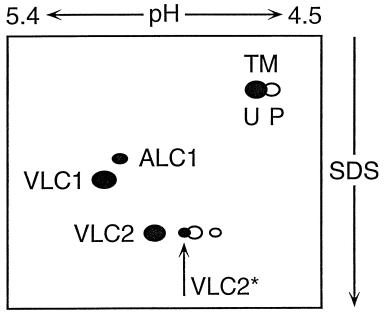

Fig. 4. Schematic drawing of the myosin light chains and α-tropomyosin (TM) resolved by two-dimensional gel electrophoresis from the ventricle of patients with hypertrophy and/or end-stage heart failure. First dimension, isoelectric focusing and second dimension in SDS with decreasing molecular mass. (U) unphosphorylated and (P) phosphorylated form of α-tropomyosin. Both VLC2 and VLC2* can be phosphorylated (large and small open symbols, respectively). Atrial ALC1 is present in addition to the endogenous VLC1 [63,68,69]. For further details see text.

Several recently detected missense point mutations in either the ELC or RLC are associated with a rare variant of FHC and with skeletal muscle myopathy [54]. In contrast to most mutations in the MHC of FHC patients which are associated with impaired motility [33], myosin from a patient with the M149V mutation in the VLC1 displayed an actin filament translocation velocity that was increased by 41% as compared to control myosin. The two mutations M149V and R154H found in the VLC1 are localized in the region where it contacts the motor domain of the MHC. This putative interaction may correspond to the base around which the regulatory domain (lever arm) moves during contraction (Fig. 3) [10]. Furthermore, three mutations in the VLC2 (A13T, E22K and P94R) all seem to be localized near the phosphorylation site at S15 [54].

5. Combinatorial associations of multiple myosin isoform subunits

It is known that the different isoforms of MHC as well as of MLC are responsible for distinct contractile properties. Therefore it is difficult to accept the idea that this isoform variety simply reflects an evolutionary relict of progredient accumulation of gene duplications, mutations and chromosomal rearrangements. The strict regulation during development and tissue-specific mode of expression seems more likely to favor the notion that the various isoforms present a store from which to choose in order to build molecular motors with functional isoform advantages. It might therefore be useful to consider the different heteromeric molecular assemblies that may possibly occur under normal and under pathological conditions.

Under normal conditions in adult human hearts the α-MHC together with ALC1 and ALC2 are confined to the atrium, while the β-MHC together with VLC1, VLC2 and VLC2* are to be found in the ventricles. Some β-MHC seems to accumulate in atria with increasing age (Table 2), which in the atrial appendages may reach values up to 50% of total MHC [55–57]. However, pressure overload models in animals as well as hemodynamic overload in humans leads to characteristic changes in myosin composition in both atria and ventricles. The ventricular myosin subunit isoforms VLC1 and VLC2 become ectopically expressed in atria in small mammals as well as in humans [31,58]. β-MHC increases in human atria up to 50% or more (Table 2) [56] and becomes reexpressed in ventricles of small animals which normally display predominantly α-MHC. In normal human ventricles a small amount of α-MHC, estimated to vary between low levels and 10%, can be observed beside the abundant β-MHC [59–62]. This residual pool of α-MHC is absent in patients under hemodynamic overload. Electrophoretic resolution of native myosin isolated from ventricles of hemodynamically overloaded small animals with varying proportions of α-MHC and β-MHC yields three species designated V1, V2 and V3 according to electrophoretic mobility. V1 and V3 consist of homodimeric αα and ββ combinations, respectively, and V2 represents the heterodimer αβ. Interestingly, myosin from overloaded atria resolves into two species only, namely A1 (αα) and A2 (ββ). A1 and A2 do not co-migrate exactly with the corresponding ventricular species V1 and V3, because they contain different MLC complements [31].

In normal human atria, two MHC homodimers (αα and ββ) together with ALC1 and ALC2 give rise to two myosin isoforms. In hemodynamically overloaded human atria, we are confronted with the co-existence of the two MHC homodimers with two types of MLC (ALC1 and ALC2 as well as VLC1 and VLC2). Both MHC homodimers may combine with each MLC type in three ways forming two homodimers and one heterodimer with regard to the MLC (ALC1/ALC1, VLC1/VLC1 and ALC1/VLC1 as well as ALC2/ALC2, VLC2/VLC2 and ALC2/VLC2). Combinatorially, this allows for $2 \times 3 \times 3 = 18$ different myosin species. Since both MLC1 and MLC2 are present in two different isoforms, the sequential arrangement along one MHC may also play a role. Two sequential arrangements are possible in one myosin molecule: VLC1–VLC2/ALC1–ALC2 as well as VLC1–ALC2/ALC1–VLC2. Therefore, one additional species has to be added for each MHC homodimer (αα and ββ) making a total of 20 instead of 18 isoforms. This number would increase even to 42 if the ventricular VLC2* were also considered. This isoform has been shown to be expressed in a proportion of VLC2* to VLC2 of around 0.4 in control as well as in overloaded human ventricles [63]. VLC2* has been shown to occur also in significant amounts in overloaded human atria [64]. In any case, multiple possible myosin isoforms could account for the altered contractility of diseased atrium [65,66] when compared to normal atrium.

In normal human ventricle, mainly β-MHC and a small amount of α-MHC beside the VLC1 and two types of MLC2 (VLC2 and VLC2*) are present. Ignoring the rare α-MHC, this would give rise to three myosin isoforms containing VLC2/VLC2, VLC2/VLC2* or VLC2*/VLC2*. As mentioned above, most of the α-MHC disappears under hemodynamic overload, leaving only the β-MHC species. However, we have reported that under such conditions the ALC1 becomes reexpressed in the ventricle varying in amounts up to 30% of total MLC1 (Fig. 4 and Table 2) [67–69]. Therefore, in diseased human ventricle the ββ-homodimer may combine with either VLC1 and/or ALC1 to form two homodimers (VLC1/VLC1 or ALC1/ALC1) and the heterodimer VLC1/ALC1. The same type of combinations are, of course, also possible with regard to the VLC2 and VLC2*. Thus, in the diseased human ventricle as many as $1 \times 3 \times 3 = 9$ myosin isoforms may co-exist. The combination with four different MLC in one molecule allows also in this case two possible sequential alignments on the MHC. This brings the total number of possible isoforms in the overloaded ventricle to 10 instead of 9.

As discussed below, shifts in expression of cardiac MHC as well as MLC are known to affect the contractile properties. In order to form heterodimeric molecules, the corresponding isoform subunits must be expressed in the same cell. This seems to be the case for ventricular myocytes as probed with monoclonal antibodies against α-MHC and β-MHC [62,70]. Atrial myocytes have also been shown to co-express α-MHC and β-MHC [55]; nevertheless, MHC heterodimers are not observed in atrial tissue. Whether or not all possible subunit associations discussed above are actually realized in vivo is not known. Functional myosin requires a strict stoichiometric relation of its subunits. The parallel occurrence of β-MHC and VLC2 in the atria of hypertensive baboons was taken to indicate a coordinated expression of myosin subunits [71]. However, cardiac MLC exhibit a slower turnover rate than MHC [72,73]. Consequently there is a pool of unassembled MLC in the cytoplasm. In accordance, we found an excess of around 0.8 molar of VLC1 over MHC in human ventricle [69]. The additional expression of ALC1 in hypertrophic ventricles added up to an excess of 3.5 molar of total MLC1 (VLC1 plus ALC1) over MHC. Isolation and analysis of myosin from such tissue indicated that, compared to VLC1, the proportional contribution of ALC1 bound to ββ-myosin was roughly the same as its content in total tissue samples. In contrast, in transgenic mice ectopically expressing the skeletal myosin ELC2fast, incorporation into cardiac myosin was not proportional to the total amount actually expressed [74]. While in atria the foreign ELC2fast replaced the endogenous ALC2 almost entirely, this hardly occurred in the ventricles, despite the fact that in both tissues αα-myosin was the recipient protein. These cases illustrate that human ventricular ββ-myosin readily combines with ALC1 and that murine atrial αα-myosin accepts skeletal muscle ELC2fast while its ventricular counterpart (also αα-myosin) does not. It may thus be emphasised that correlated regulation of expression of MHC and MLC is not required for stoichiometric assembly. In general, synthesis of MLC may be in excess over that of MHC and a large portion of the unassembled MLC will probably be degraded. On the other hand, we observed a far larger amount of β-MHC in whole tissue samples of hemodynamically overloaded human atria than VLC1 (Table 2).

A further aspect may shed some light on the question of heteromeric myosin assemblies. During development the expression of MHC and MLC isoforms undergoes transitions in atria and ventricle which are temporally not closely linked [57]. This implies a lack of isotype-specific interactions between the MHC and MLC. Taken together, the available evidence suggests that different MLC can indeed combine with different MHC. Furthermore, the MHC and MLC may be regulated independently of one another and thus allow for a variety of mixed isotype assemblies to occur in vivo.

6. Myosin cross-bridge kinetics

Cardiac contractility is directly related to the type of predominant myosin species. The motor protein defines the frame within which contractility may vary in terms of force production (unitary force per cross-bridge cycle) and velocity of displacement (kinetics). The structural and mechanical changes in the cross-bridge (a series of successive conformational changes in the myosin head domain) are coupled to a concomitant series of chemical reactions involving the hydrolysis of ATP to ADP and inorganic phosphate (confer [75,76]). The simplified scheme in Fig. 5 combines the mechanical and kinetic cycles and delineates the main intermediate states in the actin–myosin cross-bridge cycle. The affinity of myosin (M) to actin (A) changes during the cross-bridge cycle. The species with bound ATP or its products resulting from hydrolysis (ADP and Pi) exhibit low affinity, do not produce force and oscillate rapidly (on a time scale of μs or less) between attached and detached states. Consequently, they do not present significant resistance to stretch of relaxed muscle.

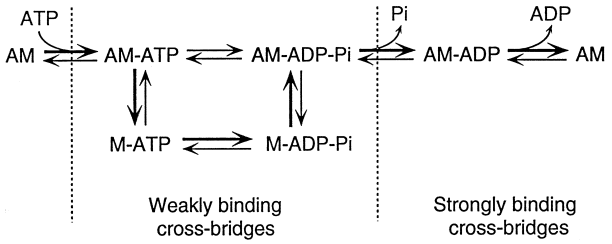

Fig. 5. Simplified kinetic scheme of the cross-bridge cycle. M, myosin; A, actin. Main reaction pathways are indicated by heavy arrows. For explanations see text.

Force production is thought to be coupled to the release of Pi and comprises the transition from weakly to strongly binding states [77,78]. These force generating cross-bridges exhibit a slower rate of oscillation between attachment and detachment bind to actin with positive cooperativity. This results in an increased resistance to passive stretch. The complete cross-bridge cycle is terminated in the nucleotide-free state after dissociation of ADP which resembles the state of rigor mortis. In vivo, upon binding of a new substrate ATP, the cross-bridge dissociates from actin and the cycle is ready to start again.

Measurements of contractile parameters such as isometric tension, shortening velocity and force redevelopment following a brief period of shortening at zero load can provide information about the distribution of cross-bridges among weakly and strongly binding states and the kinetics of transitions between them [79]. The first quantitative model which related experimental parameters to the kinetics of the cross-bridge cycle considered two states: one attached and force-generating, and one detached non-force-generating [80]. These two states are related by two rate constants: the forward rate constant f determining the rate of attachment, and the forward rate constant g determining the rate of detachment. The same model is now applied to the reaction scheme with many intermediate cross-bridge states [76]. In analogy to the original model, the rate constant f_{app} now describes the transition from weakly binding, non-force generating states, to the strongly binding, force-generating states, and g_{app} describes that for the opposite transition via product release and rebinding of ATP (Fig. 5). Since weakly binding non-force-generating cross-bridges are still in rapid equilibrium with actin [81], the rate constants no longer describe attachment and detachment, respectively. The isometric force (F) of a muscle can thus be described as

$$F = F' \cdot n_{tot} \cdot \frac{f_{app}}{f_{app} + g_{app}}$$

where F' is the force (unitary force) generated per cross-bridge and n_{tot} is the total amount of cycling cross-bridges per half-sarcomere. The expression $f_{app}/(f_{app} + g_{app})$ represents the fraction of cycling cross-bridges in the force-generating states, and hence, steady-state tension. Both an increase in f_{app} or a decrease in g_{app} will increase the fraction of force-generating cross-bridges and, therefore, increase tension generation of a muscle. The rate of transition from weakly to strongly binding states may be estimated from the rate of tension development after a brief shortening at zero load [76,82]. The rate constant of tension redevelopment (k_{td}) equals $(f_{app} + g_{app})$. The unloaded maximal shortening velocity (V_{max}) primarily depends on g_{app} which, in turn, is controlled by the release of ADP [83]. The ATPase activity during isometric steady-state tension can be described by the equation [82]

$$\text{ATPase} = n_{tot} \cdot s \cdot f_{app} \cdot g_{app}/(f_{app} + g_{app})$$

where s is the number of half sarcomeres. Tension cost is then obtained by dividing the ATPase-equation by the force-equation yielding $g_{app} \cdot s/F'$. Under isometric steady-state conditions s is constant, and if F' is equal between two myosin isoforms under consideration, then the relation of ATPase activity and tension may be directly proportional to g_{app}. An increase in g_{app} would predict a decrease in force, and vice versa.

To illustrate the complexity of the interrelation between the mechanical and kinetic properties of contractility, let us consider a muscle fiber that is stimulated to contract. The amount of force produced depends on how it is free to move. The velocity at which the fiber shortens depends on how great a load it must bear. If no load is applied, the fiber shortens at the maximal speed (V_{max}). If a force sufficient to prevent shortening is applied, the fiber develops force under isometric conditions. Whatever parameter one measures, and regardless of how it is measured, the results are not only determined by the intrinsic properties of the motor protein alone, but also by the continuously varying mechanical conditions imposed on the system by the reaction partner actin as well as by the regulatory proteins in the sarcomere. This complex ensemble is reflected in the force–velocity relation [32,79]. In performing work against a load, muscle generates power, which is work per unit of time and is obtained by multiplying force with velocity. The maximal power output depends on V_{max}, isometric tension (P_0) and on the curvature of the force–velocity curve [84]. The shape of the curve is dependent on the force coefficient a, normalised to isometric force = a/P_0. The power output is zero during shortening without load at V_{max} as well as under isometric conditions. Since force and velocity are inversely related, their product will be greatest at an intermediate load. It reaches a maximum at around 20–40% of V_{max} while at the same time producing around 30% of isometric tension.

P_0 is determined by the number of attached cross-bridges per cross-sectional area at any given time, multiplied by the force development of a single cross-bridge. The actual number of attached cross-bridges can in turn be estimated by multiplying the total number of cross-bridges (n) by the fraction of time spent in the force-generating state (duty ratio or duty cycle). V_{max} under zero load cannot be measured directly and has to be extrapolated from the force–velocity curve, or better, may be determined by Edman's slack-test [85]. Determination of isometric tension of permeabilised muscle fibers yields inconsistent results. This may be due, at least in part, to the experimental conditions (see ref. [32] for discussion). In general, the specific tension (force per cross-sectional area) does not vary much among different muscles or animal species [86,87]. In vivo, movements are seldom performed at a speed close to V_{max}. The range of speed (V/V_{max}) at which muscles mostly work, is close to the one where the maximal power output is generated [88]. Since V_{max} of fast skeletal muscle fibers is about 2–4 times higher than in

slow fibers, the maximal power output is much lower in slow than in fast fibers [89]. It has been suggested that the maximal power output may be taken as a more reliable index of dynamic mechanical properties of a muscle than V_{max} [86]. In view of the complex muscular mechanics, the diversity of myosin isoform compositions may be required to meet the physiological demands for a large number of optimal combinations between the power output and the corresponding shortening velocity in order to perform movement on an economic basis.

7. Ca^{2+}-control of cross-bridge activity

The twitch properties of fast and slow skeletal muscles depend not only on the contractile apparatus but also on the Ca^{2+}-transients in the cytosol. This is even more so in the heart. The heart muscles do not relax fully during diastole, and neither do they contract maximally during systole [90,91]. At submaximal Ca^{2+}-concentrations, the rate of tension development shows pronounced Ca^{2+}-sensitivity. The sensitivity of the myofibrillar apparatus to the activating Ca^{2+} is generally expressed by the pCa–tension relation as determined under isometric steady-state conditions on permeabilised fiber preparations. Some studies have also been carried out on intact fibers using intracellular Ca^{2+}-indicators. pCa denotes the negative decadic logarithm of the free Ca^{2+}-concentration. The normalized tension–pCa data can be fitted to the Hill equation [92]

$$Y = \frac{[Ca^{2+}]^H}{pCa_{50} + [Ca^{2+}]^H}$$

where Y is the fractional force, pCa_{50} is the Ca^{2+}-concentration resulting in half-maximal activation, and H is an index for cooperativity. The value of pCa_{50} provides an index for the affinity of Ca^{2+} to the contractile system. The coefficient H gives the minimal number of cooperating binding sites. Since cardiac troponin-C (TnC) contains only one functional Ca^{2+}-binding regulatory site, any value of $H > 1$ indicates cooperativity involving multiple complexes of the regulatory system on the actin filament (for references see [93]). Schematically, the cardiomyocyte may modulate force of contraction in two ways (Fig. 6). First, force of contraction may be altered by changing the amplitude of the cytosolic Ca^{2+}-transients. A rightward shift to higher Ca^{2+}-concentration from A to C on the abscissa causes an increase in force by shifting the degree of activation from the lower to the upper filled circle on the same intermediate tension–pCa curve (Fig. 6). Second, changes in the sensitivity of the contractile apparatus to Ca^{2+} may equally allow alterations in the force of contraction. A shift of the tension–pCa curve to the left or to the right as indicated by the horizontal arrows at a given Ca^{2+}-concentration B, will increase or decrease the degree

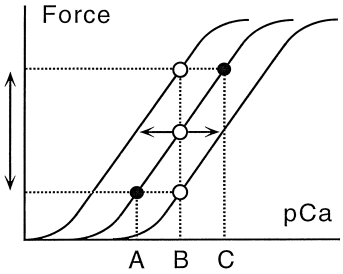

Fig. 6. Schematic illustration how the cardiomyocyte may modulate contractile force by two main mechanisms. Force is plotted versus log of increasing Ca^{2+}-concentrations. Increasing Ca^{2+} from A to C increases force in proportion without affecting the Ca^{2+}-sensitivity (filled circles on the same curve). At a given Ca^{2+}-concentration B (open circle), force can be increased by increasing the Ca^{2+}-sensitivity of the contractile system (leftward shift), or lowered by decreasing Ca^{2+}-sensitivity (rightward shift). The steepness of the force–pCa curve is a measure for cooperativity of the activation process (Hill coefficient) and is unchanged in the given examples. For further discussion see text.

of activation (open circles), respectively. The contractile force will be altered accordingly. The Hill coefficient (H) determines the steepness of the tension–pCa curve.

In general, permeabilised fibers from slow muscles exhibit a higher Ca^{2+}-sensitivity than fibers from fast muscles [94]. For activation levels up to around 20% of maximal tension, increasing Ca^{2+} seems to increase n_{tot} (recruitment of cross-bridges). At higher levels of activation, Ca^{2+} seems to increase tension by increasing f_{app}, thus affecting the cross-bridge turnover kinetics directly. These two modes of regulation do overlap, however, when going from rest to activation. The effect of Ca^{2+} on f_{app} is consistent with the observation that Ca^{2+} has only a small, if any, effect on V_{max}, since this latter is mainly determined by g_{app} which is almost independent of Ca^{2+} [76]. Thus, factors which may affect cross-bridge kinetics via f_{app} or g_{app} can become important parameters for modulation of muscle activity.

8. Effects of myosin subunit isoforms on contractility

In general, V_{max} is roughly proportional to the ATPase activity and speed of contraction in heart as well as in skeletal muscles (for reviews see [32,79]). The ATPase activity is highest in V1 ($\alpha\alpha$), intermediate in the heterodimer V2 ($\alpha\beta$) and lowest in V3 ($\beta\beta$) cardiac isomyosin of a given species [31]. In skeletal muscles the Ca^{2+}-sensitivity is about 0.2–0.3 pCa units higher in slow than in fast fibers. On the other hand, fast fibers exhibit a much steeper tension–pCa curve with an H value of 4–6, while slow fibers have one of 2–3. Human cardiac fibers from atrium and ventricle have an equally low H value of 2–3 [51]. This may have to do with the fact that the cardiac TnC is also expressed in slow skeletal muscle fibers. This cardiac TnC isoform contains only one Ca^{2+}-binding regu-

latory site instead of the two in the fast skeletal muscle TnCf [95]. In addition, the Ca^{2+}-sensitivity is 0.17 units higher in ventricular than in atrial fibers concomitant with an unloaded V_{max} (given as muscle length per second) of 2.6 for atrial and a lower one of 1.7 for ventricular fibers [51]. These characteristic differences seem to be mainly due to the atrial myosin containing mainly α-MHC as opposed to the ventricular myosin which in man contains predominantly β-MHC. Measuring the ATPase activity during isometric tension generation at various Ca^{2+}-concentrations allows to establish the ATPase-force relation for human atrial and ventricular muscle fibers [96]. Force at saturating Ca^{2+}-concentrations was 14.0 for atrial and 21.1 kN/m^2 for ventricular fibers. The amount of ATP used per force (tension cost) was independent of the Ca^{2+}-concentration and just about three times higher for atrial than for ventricular fibers. The Ca^{2+}-sensitivity was again 0.08 pCa units higher in ventricular fibers. While both V_{max} and isometric tension generation differ only moderately between atrial and ventricular fibers, the difference in the rate of tension development is quite pronounced. The rate constant of tension development (k_{td}) after photolytic release of ATP from 'caged ATP' is seven-fold higher in atrial than in ventricular porcine muscle fibers [97].

As outlined above, the ATPase-force relation is proportional to g_{app} and the force of a muscle depends on its cross-bridge kinetics. Taken together, these results suggest that atrial myosin cross-bridges differ in their kinetics from the ventricular myosin by having a higher g_{app}, implying a faster transition rate from force-generating into non-force generating states. If the unitary force value (F') were different, one fiber type would still generate different absolute tension at a given Ca^{2+}-concentration, but it should not change the normalized tension–pCa curve. A lower g_{app} in the kinetic of V3 myosin is consistent with a longer fraction of the cross-bridge cycle being spent in the force-generating states (increased duty ratio or duty cycle). This difference in kinetics between human atrial and ventricular myosin is thought to reside in the α-MHC and β-MHC. It has to be born in mind, however, that these two myosins differ in their MLC complement.

V1 and V3 myosin isoforms with the same ventricular MLC complement (VLC1 and VLC2) can be generated in rabbits by making them hypothyroid (producing V3 myosin by feeding them propylthiouracil) or hyperthyroid (producing V1 myosin by administering levothyroxine) [98]. V1 myosin has twice the actin activated ATPase activity and three times the actin filament sliding velocity in an in vitro motility assay system, when compared to V3 myosin. Yet, V1 myosin produces only half the average cross-bridge force per cycle (0.15 pN for V1 and 0.30 pN for V3 myosin). These findings also point to a kinetic difference between the α-MHC and the β-MHC in V1 and V3 myosin, respectively. Such a difference in kinetics may provide the molecular basis for the observation made earlier by myothermal techniques [99]. The economy of ATP utilisation for isometric force production (i.e. cross-bridge tension–time integral per ATP) by V3 was found to be twice that for V1 myosin. The force–time integral reflects the cross-bridge attachment time and is positively related to economy of contraction and negatively related to ATPase activity, shortening velocity and cross-bridge cycling rate. It should be noted that slower actin translocation velocities are not always associated with higher average cross-bridge force. Both V1 and V3 myosin have slower actin filament velocities than fast skeletal muscle myosin, but neither generates greater average force [100,101].

Hyperthyroid rats supplied with triiodothyronine contain exclusively α-MHC in the atria as well as in the ventricles [102]. There is no evidence that the tissue-specific MLC isoforms of atrium and ventricles undergo changes in relation to the thyroid state. The actin filament proteins do not seem to differ between these two tissues (reviewed in [102]). Thus, atrial and ventricular myocardium of hyperthyroid rats have identical composition of myofibrillar proteins except for the tissue-specific MLC. ATPase activities of myofibrils in solution (fully stimulated at pCa 4.4 and when inhibited at pCa > 8) as well as of isolated myosin are the same in atrial and in ventricular preparations, yet the contractile properties differ markedly between permeabilised fibers from atrium and ventricle. Isometric tension is 9.7 for atrial and 22.6 kN/m^2 for ventricular fibers (compare corresponding values given above for human). Maximal shortening velocity and maximal power output are almost twice as high in atrial fibers [102]. These findings suggest that in addition to the MHC also the MLC contribute to the contractile properties, while the myofibrillar ATPase activity in solution is determined entirely by the MHC species. The possible involvement of the MLC in modulation of contractility and ATPase activity has recently been suggested for skeletal muscle systems [32,46,103].

9. Modulation of contractility in hypertrophy by the myosin essential light chain

The relative composition of MHC and MLC1 isoforms in tissue samples from the free wall of all four chambers is summarised in Table 2 for six failing human hearts (five cases with dilated cardiomyopathy, DCM, and one with ischemic cardiomyopathy, ICM) obtained during transplantation and is compared to samples from five hemodynamically compensated subjects. The functional state of the patients was characterised by the New York Heart Association (NYHA) classification IV and by an ejection fraction (EF) of 16 ± 2% (normal EF > 60%). The mean pressure in the right and left atrium was 14 ± 1 (normal < 5) and 33 ± 2 mmHg (normal < 12), respectively. α-MHC and

β-MHC can be quantified after resolution by SDS-PAGE while two-dimensional gel electrophoresis is required for quantification of the MLC isoforms [68,69]. Both ALC1 and ALC2 display a higher apparent mass than the corresponding ventricular species [104]. For the ALC1 this applies only for human and pig (Fig. 4). In most other animals, including bovine, baboon and dogs, the ALC1 has a lower apparent mass than VLC1.

The change in myosin isoforms from V1 (αα) to V3 (ββ) in the ventricle of small animals under pressure overload is associated with reduced ATPase activity and V_{max}, concomitant with an increased economy of isometric force development (reviewed in [99]). The hemodynamically overloaded human atrium seems to follow a similar pattern. As mentioned earlier, the α-MHC gets partially replaced by β-MHC in human atria under hemodynamic overload (Table 2). In accordance, permeabilised fibers from hypertrophic atria of patients with various valve diseases exhibit a Ca^{2+}-sensitivity of tension 0.16 units higher and a V_{max} 44% lower than in controls [65]. Because of the close relation between MHC isoform and myocardial function, energetics and biochemistry, it is assumed that the MHC is the main determinant of the contractile characteristics.

In human ventricles however, V3 (ββ) myosin with low ATPase activity is the predominant species under normal conditions (as also in large animals such as dog, pig, bovine) and does not significantly change under hemodynamic overload. The α-MHC content of the right and left ventricles was below the biochemical detection limit both in the hemodynamically compensated and diseased hearts [67,68] (Table 2). Nevertheless, isometric tension, tension–time integral and maximal rate of tension rise are 2–3 times higher in muscle fibers from hypertrophic left ventricles of patients with severe mitral regurgitation than in controls [99]. The Ca^{2+}-sensitivity of tension was recently reported to be 0.28 units higher in left ventricular papillary muscle fibers of patients with DCM and heart failure (NYHA IV) than in samples of subjects without heart affection [105]. This shift in Ca^{2+}-sensitivity of +0.28 units is even larger than those observed between normal and diseased atrium (+0.16) or between normal atrium and normal ventricle (+0.08 and +0.17). Taken together, these findings indicate that ventricular muscle fibers from patients in end-stage heart failure behave mechanically in a similar way as do fibers from overloaded atria where α-MHC is partially replaced by β-MHC. If a change in expression of a myosin subunit isoform were responsible for the altered contractility of hypertrophic human ventricle it could only concern the reexpression of ALC1 (Table 2). The ALC2 has never been detected in normal ventricle during development, neither at high age [57], nor in diseased ventricles [68,69].

At mid-gestation the ALC1 constitutes roughly half of the total MLC1 in the human ventricle [106,107]. After birth it decreases rapidly to almost zero by the age of one year. The expression of the ALC1 persists, however, in patients with congenital heart diseases, causing an overload for the right ventricle. The persistence of ALC1 in ventricles of patients with congenital heart diseases, or its reexpression in patients with various types of heart affections, is consistently coupled with hemodynamic overload [63,106,107]. The amount of ALC1 in the ventricle may vary from a few up to over 30% of total MLC1. In 16% of over 100 patients with hemodynamic overload ALC1 was not detected in the ventricle.

The results of mechanical tests with permeabilised fibers from patients containing from zero up to 20% ALC1 are summarised in Fig. 7 (data taken from Refs. [63,107]). Material without detectable ALC1 was included from subjects with normal hemodynamics. We showed for the first time, assuming other things being equal, a positive linear correlation between V_{max} and Ca^{2+}-sensitivity of isometric tension versus the ALC1 content. The correlation coefficient r for V_{max} (12 cases) and Ca^{2+}-sensitivity (8 cases) is 0.82 and 0.92, respectively ($p < 0.01$ in both cases). Thus, both these two mechanical parameters are proportional to the ALC1 content. If taken together, the combined regression line in Fig. 7, would have a correlation coefficient of 0.86 with a significance level of $p < 0.001$. An increase of the Ca^{2+}-sensitivity by +0.36 units and an increase of V_{max} by a factor of 1.7 can be derived from the regression for an increase of 20% ALC1 in the ventricular tissue. In addition to V_{max}, the curvature of the force–velocity relation changes as indicated by a decrease of the normalised tension coefficient a/P_0 from 0.21 in fibers

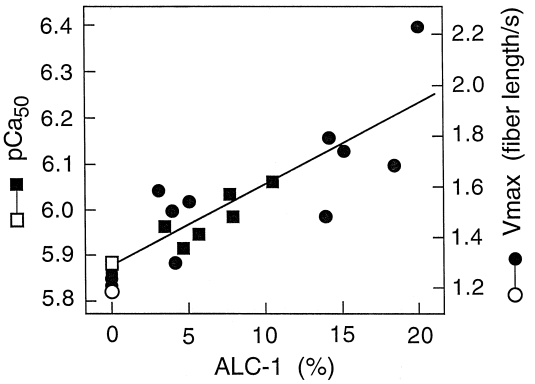

Fig. 7. Correlation of Ca^{2+}-sensitivity of tension (pCa_{50}, Ca^{2+} required for half-maximal force generation) and maximal shortening velocity (V_{max}, expressed as muscle length per second ML/s) versus ALC1 content (expressed as % of total MLC1 isoforms) assessed on permeabilised ventricular muscle fibers. The regression line for pCa_{50} of left ventricular papillary muscle fibers from seven patients with end-stage heart failure (filled squares) and one control (open square) has a correlation coefficient r of 0.92 ($p < 0.01$). The regression line for V_{max} of right ventricular infundibular muscle fibers from 11 patients with congenital heart diseases (filled circles) and one control (open circle) has a correlation coefficient r of 0.82 ($p < 0.01$). The combined r for all 20 cases would be 0.86 ($p < 0.001$). Data taken from Refs. [63,107]. For further discussion see text.

lacking ALC1 to 0.15 in fibers containing 20% ALC1 [107]. The rate of tension development is almost twice as fast in fibers with high ALC1 when compared to fibers with low ALC1 content. Isometric tension at saturating Ca^{2+}-concentrations was 352, 660 and 835 kN/m for fibers with zero, 3–5% or with 20% ALC1, respectively. All muscle specimens were treated with cardioplegic solution prior to chemical skinning and the MLC2 were thus fully dephosphorylated [63,107].

Taken together, these findings indicate that ALC1 modulates cross-bridge kinetics and thus contractility in human ventricular muscle. Increasing ALC1 increases V_{max} which is proportional to g_{app} as well as the rate of tension development k_{td} which equals the sum of $(f_{app} + g_{app})$. ALC1 thus accelerates the cycling kinetics. This may be brought about by increasing either f_{app} or g_{app}. Which one occurs with ALC1 cannot be decided from the measurements of V_{max} and k_{td} [107]. Either change would affect the fraction of force-generating cross-bridges, $n = f_{app}/(f_{app} + g_{app})$. The Ca^{2+}-sensitivity of isometric tension would, however, be affected in opposite direction by an increase in either f_{app} (increasing it) or g_{app} (decreasing it) [76,82]. In view of the positive correlation with an increase in Ca^{2+}-sensitivity (Fig. 7), one may conclude that ALC1 increases cross-bridge kinetics by accelerating selectively f_{app} over g_{app}. Even if both rate constants were increased in the presence of ALC1, f_{app} should be accelerated significantly more than g_{app}. This results in a ββ-myosin cross-bridge with ALC1 having a longer duty cycle (fraction of time spent in force-generating states per ATP-cycle) in conjunction with faster kinetics than normal ventricular ββ-myosin with VLC1. These kinetic characteristics are compatible with the mechanics described for ventricular muscle fibers from patients with severe mitral regurgitation (mentioned above) for which the ALC1 content was not known [99]. Assuming constant unitary force F' of ββ-myosin with either MLC1, the species containing ALC1 with a longer duty cycle would represent an economic improvement over that with VLC1.

10. Molecular mechanism

The molecular mechanism for MLC1 to affect the cross-bridge kinetics seems to reside in its Ala–Pro-rich extended N-terminus which has been shown to interact with the C-terminus of actin [41,42,44]. The first hint that the MLC1 might affect the actin–myosin interaction and contractility came from experiments with skeletal muscles (reviewed in [45]). In vertebrate fast skeletal muscles, two isoforms of the MLC1 (ELC1fast and ELC2fast) are expressed which result from different transcription sites and differential splicing of the same gene [108]. The ELC2fast is missing the N-terminal 42 amino acid residues and does not bind to actin. V_{max} is twice as high for rabbit fast psoas muscle fibers reconstituted with ELC2fast than with ELC1fast [45]. A similar difference in translocation velocity between myosin with either ELC2fast or ELC1fast is also observed in the in vitro motility assay system [109].

A number of key experiments shed some light on the molecular mechanism how the MLC1 modulates contraction in cardiac muscle. Human ALC1 can be reconstituted to rabbit fast skeletal muscle myosin subfragment-1 (S1) for probing its effect on the actin activated ATPase activity. Incorporated into S1, the ALC1 can also be chemically crosslinked to actin [43,47]. After removal of the first 11 residues by genetic engineering, the ALC1 can no longer be crosslinked to actin and the ATPase kinetics resemble those of the S1-actin complex with the short ELC2fast isoform. In conclusion, the first 11 residues at the N-terminus of the human ALC1 are sufficient to interact with actin and to modulate the ATPase kinetics. It is likely that this also applies to the human VLC1 as well as to the other MLC1 types. Indeed, removal of the first 13 residues from ELC1fast of chicken skeletal muscle myosin also converts the ATPase kinetics of the S1-actin complex with ELC1fast to those of S1 containing the short ELC2fast [110]. The 13-residue peptide 1-APKKDVKKPAAAA-13 was removed by proteolytic cleavage with papain and is identical in sequence to the corresponding N-terminus of the rat ELC1fast (Table 3). In particular, it contains the N^{α}-trimethylalanine at its N-terminus like all MLC1 beginning with Ala [111]. This 13-residue peptide can itself be crosslinked to actin and, when added to the S1-actin complex containing the endogenous intact ELC1fast, it increases the apparent Michaelis constant (K_m) as well as the rate of ATPase activity. This was interpreted as indicating that the binding of the 13 residue peptide to actin prevents the normal interaction of the endogenous ELC1fast of S1 with actin and thus converts its ATPase kinetics to those of S1 with the short ELC2fast. Interestingly, a synthetic decapeptide 1-APKKDVKKPA-10 missing the N-terminal N^{α}-trimethyl group had hardly any effect on the ATPase kinetics [110].

All MLC1 given in Table 3 comprise four positive charges within the first nine residues distributed over two couples of Lys with one negative charge (Asp or Glu) in between. Removal of the positive charges in Lys3 and Lys4 by exchanging them for Ala by site directed mutagenesis in ALC1 also affects the ATPase kinetics approaching those of an S1-actin complex with the short ELC2fast [43]. Changing the first two Lys3 and Lys4 to Ala or all four, including also Lys7 and Lys8, leads to a gradual increase in V_{max} of rabbit fast psoas muscle fibers after incorporation of the mutated rat ELC1fast species in comparison to fibers with its wild type ELC1fast [45]. This demonstrates that the four positive charges among the first nine amino acid residues of MLC1 are involved in long-range electrostatic interactions (salt bridges) with the C-terminus of actin. The C-terminus of actin does, indeed, contain three negative charges in the positions 12, 13, and 15 from the end (Table 3). Cross-bridges with MLC1

containing the characteristic N-terminal extension, display a relatively slow cycling rate in comparison to the short ELC2fast. Weakening these electrostatic interactions while leaving the lengths of the extended N-terminus of the MLC unchanged accelerates cross-bridge kinetics. Taken together, these findings indicate that all long MLC1 species attach with their extended N-terminus to actin and may exert a mechanical drag which slows down the kinetics of the cycling cross-bridge.

In contrast to ELC1fast, ALC1 contains one additional negative charge (Asp) following immediately after the second couple of Lys, while two negative charges (Glu) follow in the VLC1 (Table 3). The first nine residues in human ALC1 and VLC1 are identical in sequence. VLC1 contains Glu at position 9 instead of Asp representing a conservative exchange. An additional negative charge follows, however, in VLC1 with Glu-11. It was recently shown in an in vitro binding assay that a decapeptide corresponding to the residues 4–13 of human ALC1 binds with half the affinity to actin than the corresponding decapeptide from VLC1 [112]. The weaker binding of the ALC1 decapeptide is paralleled by faster cross-bridge kinetics in ventricular fibers with ALC1. These results imply that not only the positively charged residues of the MLC1 N-terminus interact with actin, but that also the additional negative charges in the cardiac MLC1 (not present in skeletal muscle ELC1fast) may differentially interact with the two positive charges near the end of the C-terminus of actin (Table 3).

The synthetic decapeptide 4-KPEPKKDDAK-13 of human VLC1 was used to test its effects on human cardiac muscle fibers [113]. It enhances V_{max}, rate of tension development and rate of relaxation when diffused into intact atrial fibers. These effects are not associated with modulation of intracellular Ca^{2+}-transients as monitored by the fluorescence of the Ca^{2+}-indicator Fura-2. It further increases isometric tension at submaximal (pCa 5.5) and maximal (pCa 4.5) Ca^{2+}-activation of permeabilised human ventricular papillary muscle fibers [113]. In addition, this human VLC1 decapeptide induces a supramaximal increase in the ATPase activity of rat ventricular myofibrils at submaximal Ca^{2+}-levels with no effect at low (pCa below 7.0) and at maximal Ca^{2+}-levels (pCa above 5.0) [114]. When troponin-I (TnI) and TnC were extracted from the myofibrils, the VLC1 decapeptide lost its ability to stimulate the ATPase rate. This effect was fully restored upon reconstitution with the TnI-TnC complex [114]. Thus, the activation of the myofibrillar ATPase activity by the VLC1 decapeptide requires a full complement of the actin filament regulatory proteins (troponin-I, -C, -T and tropomyosin). Interestingly, the stimulatory effect occurs at a ratio of 4 peptide molecules per one entire actin filament of roughly 1 μm length and containing approximately 400 actin monomers, suggesting that the peptide engages in a highly cooperative process. Based on these findings it was proposed that at a submaximal level of Ca^{2+} the peptide analogue allows bound cross-bridges to turn 'on' more actin monomers (or actin filament functional units) as opposed to without it [114]. The cooperative response may be the result of peptide-induced alterations in the interactions between the troponin complex, tropomyosin and the actin strands which can spread throughout most, or even all, of the actin filament.

It has been hypothesized that the primary function of the extended MLC1 N-terminus may be to provide a tether between the myosin and actin filaments. Such a tethering would serve to position the two filament systems for cross-bridge interaction [45]. This could be more important for sarcomere assembly during development than in mature striated muscles. This, in turn, may be reflected by the lack of expression of the short ELC2fast in early muscle development. The extended N-terminus is required to bridge the 8 nm gap between the bulk of the MLC1 and the actin surface. Due to the particular sequence rich in Ala and Pro the N-terminus is elongated and rigid prior to its binding to actin (reviewed in [47]), If it were flexible and would assume a globular structure in the detached state, actin binding would be accompanied by a transition from a folded to an extended form. Such a transition would be energetically unfavorable (breaking of intramolecular contacts and imposition of greater order to the system) [115]. The binding site at the end of an extended arm, as opposed to a flexible structure, may also enhance the association rate with actin, but leaving the dissociation rate unaffected [47].

Finally, myosin binds with its upper and lower portion of the 50-kDa segment to two adjacent monomers in the actin filament (Fig. 3) [23]. The MLC1 arm may then reach to the second actin which is also in contact with the lower portion of the 50-kDa, although it has not been demonstrated yet which of the actins interacts with the MLC1. There are then two pathways by which information could be transmitted to the active site which is about 8 nm distant from the actin filament [23,44], one proceeding via the MHC in the head portion, and the other passing through the extended MLC1 arm and travelling backwards up the motor domain [47]. Taken together, these findings give new insight into the molecular complexity and subtlety of the actin–myosin interaction and its modulation of the cross-bridge kinetics by the MLC1 not anticipated before.

11. Pathophysiological significance of myosin essential light chain expression in cardiac hypertrophy and failure

The human ventricular muscle contains ββ-myosin with low ATPase activity but which performs work at a higher economic level with regard to ATP utilisation in compari-

son to the faster contracting atrial αα-myosin. Under hemodynamic overload, the type of MHC in the ventricle does not, in contrast to atrial muscle, change. However, the fetally expressed ALC1 becomes reexpressed in the ventricle. This ALC1 modulates ventricular contractility by accelerating the cross-bridge kinetics of ββ-myosin and at the same time, it improves the economy by prolonging the duty ratio or duty cycle. The most significant consequence may be the increase in Ca^{2+}-sensitivity of the contractile apparatus containing ALC1. This is particularly important since myocardial contractility is governed by moderate fluctuations in cytosolic free Ca^{2+}-concentrations between systole and diastole [90,91]. Little or no tension is seen at around 0.1 μM free Ca^{2+} in the cytosol. Under rest the heart operates only at about 25% of maximal activation (reviewed in [116]). Thus force can increase considerably in response to increasing Ca^{2+}-transients, even if the Ca^{2+}-sensitivity of the myofilaments remains unchanged. The reexpression of ALC1 in the ventricle improves contractility and can be seen as part of the adaptive response during hypertrophy. The extent of ALC1 expression in patients with different types of cardiac hypertrophy varies considerably [68,69,117,118]. The observation that the extent of ALC1 accumulation correlates with the degree of hemodynamic load the heart has to bear, but not with any other parameter such as muscle mass or non-muscle tissue content, points to its physiological relevance [68]. Consequently, the ALC1 content was indeed reduced after successful surgical interventions which lowered the hemodynamic overload in patients with aortic valve diseases [69].

Although muscle specimen are often obtained from patients with end-stage heart failure who underwent cardiac transplantation, the ALC1 content is by no means higher in the ventricles of these hearts than in patients in the compensated phase of hypertrophy (Table 2) [63,68,69,105,107]. The question arises, however, whether the increased contractility induced by ALC1 may turn into maladaptation in heart failure. Heart failure is characterised by decreased contractility and a low ejection fraction (around 30% or lower). A number of observations made recently, may be at the heart of the matter. Of foremost importance is the handling of intracellular Ca^{2+}. A number of proteins involved in intracellular Ca^{2+}-homeostasis have been shown to be lower in end-stage failing compared to stable cardiac hypertrophy: calsequestrin (Ca^{2+}-binding protein of the sarcoplasmatic reticulum, SR), the Ryanodine receptor (Ca-release channel of SR), SERCA2 (SR-Ca-ATPase) and phospholamban (regulatory protein of SERCA2) [119]. In contrast, the Na–Ca-exchanger is increased [120]. Thus, more Ca^{2+} is extruded by the Na–Ca-exchanger instead of being sequestered by the intracellular SR, and as a sequel, the myocytes in the failing heart become impoverished on Ca^{2+}. Consequently, much lower systolic Ca^{2+}-transients are seen in cardiomyocytes from human hearts in end-stage failure [121]. This, in turn, results in blunting the normally observed frequency potentiation of contractile force [122–124]. The heart responds with enhanced frequency to the higher catecholamine levels during hypertrophy, but lacks the ability to increase contractile force. The cytosolic Ca^{2+}-levels are only moderately increased during diastole in failing hearts [121] and hardly affect diastolic tension [125]. In addition, it was recently shown in the spontaneously hypertrophic rat model that a defect in excitation–contraction coupling (the Ca^{2+}-induced Ca^{2+}-release from SR) is compensated for by the increased level of circulating catecholamines [126]. Failing of this compensatory mechanism due to a down-regulation of β-adrenergic receptors [127] is thought to be causally linked to the onset of heart failure in this model. The presence of ALC1 then still represents an advantage even during heart failure, in that it allows for a faster and larger force development at decreased systolic Ca^{2+}-levels in compliance with a high heart rate.

On the other hand, inadequate blood supply may often be associated with heart failure. Under such conditions, the energy demand of the hypoperfused myocardium exceeds the reduced energy supply. The myocardium reacts to this imbalance by reducing its contractile function. The mechanism responsible for the rapid reduction in contractile function during myocardial ischemia remains unclear [128,129]. Teleologically speaking, it seems to represent an attempt to match energy demand with supply. This perfusion–contraction matching may be termed myocardial stunning or hibernation [116,130]. The myocardium may recover without loss of function [131]. If it fails, however, to reach perfusion–contraction matching, myocardial infarction develops. Under normal conditions, 15 to 30% of the total energy expenditure is consumed by non-cross-bridge processes, mainly by ion pumps (sarcolemmal Na-K-ATPase and SERCA2) to maintain the Ca^{2+}-homeostasis [1,132]. This part of energy consumption becomes prominent in the hibernating myocardium. An insufficient energy supply may thus affect the membrane stability and lead to an uncontrolled increase in cytosolic Ca^{2+}. Cytosolic Ca^{2+}-levels of 2–75 μM have been shown to occur in ferret cardiomyocytes, at least transiently, during ischemia and post-ischemic reperfusion [133]. Any increase in cytosolic Ca^{2+} above threshold level of contractile activation during diastole causes diastolic dysfunction. This is characterised by a lack of sufficient relaxation and a build-up of the preload. Due to reduced chamber filling, the ejection fraction may diminish. If the myocardium contains ALC1, its increased Ca^{2+}-sensitivity accentuates diastolic dysfunction and thus turns the originally beneficial adaptation into maladaptation.

Elevated cytosolic Ca^{2+}-levels may, in addition, activate non-lysosomal neutral proteinases such as μ-calpain (Ca^{2+}-dependent proteinase-I) which requires 1–100 μM Ca^{2+} for activation [134]. There are a number of sarcomeric proteins which are selectively susceptible to such neutral proteinases and which have been shown to be

degraded in terminally failing hearts. The giant protein titin (3000 kDa) which spans the entire half sarcomere by connecting the myosin filament with the Z-line [135] is reduced in mRNA and protein content in failing human hearts [136]. This is accompanied by the occurrence of a titin with lower molecular mass, which probably represents a degradation product. At the same time, myofibrillar and cytoskeletal structures are impaired on the immunocytochemical and electron microscopic level [137–139].

The selective degradation of the VLC2 has been reported for patients with DCM undergoing heart transplantation [140]. Myosin isolated from these specimens displayed a lower actin activated ATPase activity and an impaired capability to assemble into synthetic myosin filaments in vitro. It was further shown that these hearts contained an increased proteolytic activity specific for VLC2 [140]. Removal of RLCfast has been shown in skeletal muscle fibers to slow down V_{max} and to shift the pCa–tension curve to the left (increasing Ca^{2+}-sensitivity) [141,142]. It was concluded that removal of RLCfast slows down g_{app} and increases, therefore, the number of force generating cross-bridges. Whether degradation of VLC2 is typically associated with failing DCM hearts has not been established unequivocally. We did not observe a change in the stoichiometry of the MLC1 and MLC2 complement in terminally failing hearts of patients with DCM or ICM [63,136].

It has recently been shown in the rat experimental model that TnI is selectively degraded in the stunned myocardium [143]. This degradation can be prevented when stunning is followed by low Ca^{2+}-reperfusion. Addition of μ-calpain (calpain-I) to permeabilised myocardial fibers produces identical digestion patterns. It is hypothesized that the Ca^{2+}-activated proteinase cleaves TnI in ischemic myocardium and that this may directly impair contractility. Ischemia and hibernation are, however, complex processes in which numerous factors contribute to the pathophysiology. Whether global or locally restricted, their repercussions will affect, and most probably, will precipitate the course of heart failure.

12. Other mechanisms modulating contractility

Changes in isoform expression of sarcomeric proteins other than the MLC1 may also affect Ca^{2+}-sensitivity of the contractile apparatus. The cardiac troponin (Tn) complex comprises the inhibitory (TnI), the Ca^{2+}-binding (TnC) and the tropomyosin binding (TnT) subunits. Four of the 36 potential splice variants of TnT [144,145] have been found in human hearts [146,147]. Their appearance is developmentally regulated and TnT3c predominates in adult human hearts. Reexpression of the fetal TnT4c isoform has been observed on the mRNA and protein level in some few cases of end-stage heart failure [145–148]. The occurrence of TnT4c was associated with decreased myofibrillar ATPase activity in human hearts. The Ca^{2+}-sensitivity of tension is also lower in permeabilised heart muscle fibers of rats and rabbits when TnT4c is present [149,150]. Taken together, the fetal TnT4c isoform has the opposite effect on Ca-sensitivity than ALC1. It is not known whether the expression of TnT4c correlates to the hemodynamic load. If its expression were triggered by mechanical load as that of ALC1 seems to be, both TnT4c and ALC1 would increase concomitantly and antagonise each other's effect on contractility. Since mechanical data correlate linearly with the ALC1 content (Fig. 7) the possible negative effects of TnT4c, whatever its actual concentration, would be minor. It could be that a variable degree of expression of TnT4c contributes to the scatter of the data.

In both normal and diseased human hearts, we found only cardiac TnI [63]. Slow skeletal muscle TnI (TnIs) mRNA may be present in infants but not in adult human hearts [151]. TnIs has been shown to increase Ca^{2+}-sensitivity of cardiac myofibrillar ATPase activity in comparison to cardiac TnI [152]. The two isoforms of TnC (TnCf in fast skeletal muscles and TnCs in heart and slow skeletal muscles) originate from two different genes [153]. Only TnCs is found in the heart. Replacement of TnCf by TnCs reduced the Ca^{2+}-sensitivity of tension in fast skeletal muscle fibers in vitro [154]. This transition is accompanied by a decrease of the Hill coefficient from 7.7 to 2.4. In contrast, the Hill coefficient remains unchanged around 2 with increasing ALC1 content and increasing Ca^{2+}-sensitivity in failing human hearts [63]. If sufficient TnCf was expressed in failing hearts to positively affect the Ca^{2+}-sensitivity, the Hill-coefficient would be expected to rise concomitantly.

Improved contractility has been shown in BALB/c mice which express skeletal muscle α-actin in their hearts in comparison to normal mice with mainly cardiac α-actin [155]. In human hearts both cardiac (~ 40%) and skeletal (~ 60%) α-actin isoforms are expressed. However, this ratio does not change in patients with DCM or ICM as indicated by mRNA levels [156]. Ectopic expression of the β-tropomyosin (β-Tm) in the heart of transgenic mice produces an increase in Ca^{2+}-sensitivity and a decrease in the rightward shift of the pCa–tension relation induced by cAMP-dependent phosphorylation of TnI (see below) as opposed to the α-Tm isoform which is normally present [157]. In human myocardium we find almost exclusively the α-Tm isoform and no change in patients with compensated hypertrophy [69] nor in end-stage heart failure [63].

Familial hypertrophic cardiomyopathy (FHC) constitutes a genetically heterogeneous disease. The hypertrophy seems to be a reactive adaptation to the underlying disturbance of the contractile apparatus which is caused mostly by missense or deletion mutations in any of the following sarcomeric proteins: MHC, VLC1, VLC2, α-Tm, cardiac MyBP-C or cardiac TnT (reviewed in [5,27,54]). Most of these mutated proteins are functionally dominant-negative

by reducing contractility and motility in in vitro assay systems.

A number of short-term modulatory mechanisms involving reversible phosphorylation (Table 4) affect cardiac contractility in addition to the long-term adaptive changes in gene expression. Human VLC2 (164 amino acids) contains near its N-terminus (1-APKKAKKRAGGANSNVF-17) one recognition site for phosphorylation of the Ser14 by either myosin light chain kinase (MLCK) or protein kinase-C (PKC) but not by protein kinase-A (PKA) [128,158,159]. The three basic residues (KKR) upstream of Ser14 and the highly conserved three residues following Ser14 (NVF) constitute the substrate specificity for MLCK. In addition, all vertebrate RLC contain the two adjacent Lys (K) near their N-terminus. Thus the clusters of positive charges are reminiscent of those in the MLC1 isoforms (compare Table 3). These positive charges near the N-terminus may in both cases facilitate to contact the binding site of the reaction partner: (i) the proximate actin monomer in the case of MLC1 (as discussed above) and (ii) MLCK in the case of MLC2. Phosphorylation of both VLC2 and ALC2 affects contractility by increasing the Ca^{2+}-sensitivity of force development as well as ATPase activity [97,160–163]. This seems to be achieved by an increase in the rate f_{app} of the transition from non-force to force-generating cross-bridges [97]. ALC2 can also be bis-phosphorylated, however, the physiological significance of this feature is not known [53]. Sequence data are not yet available for ALC2.

Phosphorylation of TnI by PKA which results from β-adrenergic stimulation, leads to a decrease in the sensitivity of the contractile proteins to Ca^{2+} which is accompanied by an increase in cross-bridge cycling rate (reviewed in [128]). Cardiac TnI is thought to exert its effects by reducing the affinity of TnCs for Ca^{2+}. This may function as a negative feed-back mechanism by accelerating the relaxation rate of the beating heart under the influence of β-adrenergic stimulation [164]. Human cardiac TnI contains 209 amino acids with a 31 residue extension at its N-terminus found neither in the fast nor in the slow skeletal isoforms [165]. Phosphorylation of the two adjacent Ser22/Ser23 is required for these effects (Table 4) [166]. This represents an interesting situation where the tissue-specific hormonal response is discriminated not at the receptor level at the cell surface, but inside the cell by a particular amino acid sequence in the target protein which is not present in the isoforms of other muscle tissues. The same principle applies also to the cardiac isoform of the myosin binding protein-C (see below). In addition, cardiac TnI as well as TnT can be phosphorylated at multiple sites by PKC isoforms (Table 4). In general, phosphorylation of either cardiac TnI or TnT by PKC isoforms reduces Ca^{2+}-sensitivity and actin activated ATPase activity [167].

The α-Tm can also be phosphorylated at its penultimate Ser283 which enhances the actin activated ATPase activity without changing the Ca^{2+}-sensitivity [168]. This phosphorylation site lies in the region where two adjacent Tm molecules join head-to-tail together by overlapping with about 9 residues. The N-terminal portion of TnT which connects the Tn complex to Tm also makes contact in this region [168]. Responsible for α-Tm phosphorylation is a Tm kinase which does not require Ca^{2+}, calmodulin nor cAMP [169]. The myosin binding protein-C (MyBP-C, 137 kDa) (Figs. 1 and 2) is the only protein in the myosin filament that can be phosphorylated by PKA [128,170]. Human cardiac MyBP-C (1173 amino acids) contains a nine-residue specific loop 174-LAGGGRRIS-182 in the N-terminal region which can be reversibly phosphorylated upon β-adrenergic stimulation and which is not present in the skeletal muscle isoforms [171]. As a consequence, the cardiac MyBP-C can be phosphorylated at four sites in contrast to only one such site in the skeletal muscle isoforms (Table 4). One of the four sites in cardiac MyBP-C (Ser182 located in the cardiac-specific loop) needs to be phosphorylated first by a Ca^{2+}-calmodulin dependent protein kinase (CAMK) before PKA is able to phosphorylate the remaining three sites (Ser173/Ser202/Thr261) [171]. MyBP-C can also be phosphorylated by PKC. Both PKA and PKC have been shown to phosphorylate the same sites

Table 4
Phosphorylation of cardiac sarcomeric proteins by various protein kinases. Phosphorylation sites are given where known and numbered according to human protein sequences. These phosphorylation reactions have been shown to occur in adult cardiomyocytes. For discussion see text

Protein kinase	Cardiac protein	Phosphorylation sites	Ca-sensitivity of contractility or activation of ATPase activity	Ref.
MLCK/PKC	VLC2	Ser14	↑	[159]
PKA	TnI	Ser22/Ser23	↓	[167,185]
PKC-α	TnI	Ser41/Ser43/Thr142	↓	[167,185]
PKC-δ	TnI	Ser22/Ser23/Ser41/Ser42/Thr142	↓	[167,185]
PKC-ζ	TnT	multiple sites	↓	[185]
Tm kinase	α-Tm	Ser283	↑	[168,169]
CAMK	MyBP-C	Ser182	?	[171]
PKA/PKC	MyBP-C	Ser173/Ser202/Thr261	↑	[170–172]

on cardiac MyBP-C [172]. Phosphorylation of MyBP-C provokes the cross-bridges to extend from the backbone of the myosin filament [170]. This could facilitate cross-bridge attachment to the actin filament and thereby modify the kinetics of force generation in activated cardiac muscle. Its specific effect on contractility is not known at present, since no method exists for removal and reconstitution of MyBP-C in isolated contractile systems. In situ phosphorylation of MyBP-C cannot be controlled selectively without affecting other sarcomeric proteins as well.

Finally, cardiac contractility is also influenced by changes in sarcomere length and variations in intracellular metabolites. Lowering of intracellular pH and accumulation of inorganic phosphate Pi are known to lower Ca^{2+}-sensitivity of force. It has recently been shown that the pH-dependent influence on the Ca^{2+}-sensitivity is mediated by TnI which modulates the binding affinity of Ca^{2+} to cardiac TnC [173]. During hypoxia accumulation of intracellular Pi may reach levels higher than 5 mM [174]. This leads to reduced tension generation at unchanged or increased intracellular Ca^{2+}-transients [175,176]. In normal heart muscle as well as during hypertrophy the Ca^{2+}-sensitivity significantly increases with stretch, i.e. with increasing sarcomere length. This phenomenon provides the basis for the Frank–Starling mechanism which is one of the most important regulatory devices in controlling cardiac contractility from beat-to-beat. The length-dependent change in Ca^{2+}-sensitivity of permeabilised cardiac muscle fibers correlates more closely to the change in interfilament spacing than to the change in sarcomere length [177,178]. The narrowing of the interfilament lattice spacing during lengthening seems to facilitate cross-bridge attachment to the actin filament. Replacement of endogenous cardiac TnC by the skeletal muscle isoform of TnC results in a reduction of the length dependence of cardiac fibers to the level of skeletal muscle fibers [179]. The lowered length dependence can be reversed to the original cardiac level by reconstitution with a TnC chimera containing the first 41 amino acid residues of cardiac TnC spliced onto the skeletal muscle isoform. Ectopic expression of skeletal muscle TnC in the hearts of transgenic mice did, however, not change the Ca^{2+}-sensitivity of tension in these hearts [177]. There is some controversy in the literature whether or not this mechanism is operative in the failing heart. Left ventricular papillary muscle strips from patients with DCM and heart failure failed to increase force upon increasing preload when electrically driven at 1 Hz and 37°C, as did those from non-failing hearts [105]. Permeabilised muscle fibers from the same failing hearts displayed a higher Ca^{2+}-sensitivity than controls, a sensitivity however, which did not further increase under pre-stretch. This is in contrast to a recent study in which an unchanged positive Frank–Starling mechanism was demonstrated in hearts of patients with end-stage failure suffering from DCM or ICM [180]. Mechanical tests were done on whole left ventricles, on isolated intact muscle strips and on permeabilised fibers. The reason for the different results is not clear. One wonders, whether such differences may be due to degradation processes either in the contractile or cytoskeletal structures stemming from in vivo stages or occurring during sample preparation.

13. Conclusions and outlook

We have first reported that the reexpression of ALC1 in human ventricle during hypertrophy is related to the degree of hemodynamic load [68,69]. We are now able to explain the underlaying molecular mechanism for the improved ventricular contractility by ALC1. ALC1 enhances cross-bridge kinetics in such a way that force generation is increased at a given cytosolic Ca^{2+}-concentration. This functional effect can be traced to the particular primary structure of the ALC1 which modulates the interaction of myosin with the actin filament during the cross-bridge cycle.

In addition, covalent protein modification by reversible phosphorylation may transiently affect contractility, but it does so in a complex manner. Increased β-adrenergic stimulation is characteristic for hypertrophy as well as for heart failure. This stimulation mainly operates via the adenylate cyclase/cAMP/cAMP-dependent PKA system (reviewed in [8]). Increased PKA activity is known to induce phosphorylation of (i) Ca^{2+}-channels in the sarcolemma, (ii) phospholamban (regulatory protein of SERCA2) and (iii) sarcomeric proteins TnI and MyBP-C (reviewed in [181]). This results in a higher intracellular Ca^{2+}-level. The phosphorylated TnI attenuates contractility. The increased Ca^{2+} (together with calmodulin) stimulates the activity of MLCK and increases MLC2 phosphorylation. Phosphorylated MLC2 promotes contractility and thus counteracts the attenuating effect of phosphorylated TnI. Ca^{2+}-calmodulin is also required for activation of CAMK which, in turn, allows for the sequential phosphorylation of MyBP-C by PKA which may also enhance contractility. On the other hand, a number of G-protein coupled receptor systems (including muscarinic cholinergic, angiotensin-II, endothelin-1 and α-adrenergic) in the cardiomyocyte induce PKC activation [8,172]. PKC phosphorylates TnT and TnI at various sites depending on the isoform species. PKC furthermore, competes with MLCK and PKA for phosphorylation of the same sites in MLC2 and MyBP-C, respectively (Table 4). The interconnection between different signaling pathways involving various kinds of protein kinases which may result in identical phosphorylation of several target proteins, seems at first sight to comprise some redundancy. However, in view of the multifactorial etiology that leads to hypertrophy and heart failure, the cardiomyocyte depends on a complex

repertoire of mechanisms for modulating and fine-tuning its contractile responses to varying demands.

In conclusion, the short-term regulatory processes of cardiomyocytes may vary rapidly with time in response to global changes in the whole body or to local changes in the heart muscle. These rapid changes may enhance or restrict contractility in accordance with temporary demands and energy supply. The adaptive long-lasting changes in gene expression provide the basis the short-term modulatory processes may act upon. The particular state in which the myocyte finds itself, thus, determines whether the increased Ca^{2+}-sensitivity of the contractile apparatus brought about by the expression of ALC1 in the hypertrophic ventricle may be beneficial or detrimental in the event of heart failure.

In the near future we will have to learn more about molecular mechanisms of normal and compromised heart function. This may be pursued by two main routes of research involving (i) transgenic animals with either ablation or overexpression of specific genes, and (ii) primary cultures of cardiomyocytes and other heart cells as an in vitro model system for the hypertrophy reaction. The first approach gives information on functional alterations in the intact organism with all its complex responses and compensatory mechanisms. The second approach allows to study the effects of single hypertrophic stimuli (such as hormones, growth factors, cytokines, vasoactive peptides as well as mechanical stress) under defined conditions on cell morphology as well as on gene expression [5,7,8]. Today, primary culture systems still have to be used as long as no viable established myocardial cell lines exist. This arises from the fact that the cell cycle of the terminally differentiated cardiomyocytes is blocked. A further goal in heart research will certainly be to overcome this block by interference with the cell cycle control mechanisms. This could eventually open the possibility to compensate cardiovascular overload by controlled hyperplasia (proliferation of cardiomyocytes) rather than hypertrophy alone. As long as this route is barred, it seems desirable to better control the hypertrophy reaction by furthering the positive and minimizing the negative aspects. To this end, the gene expression pattern needs changing. That may be achieved by either administering drugs such as cell mediators and anti-sense oligonucleotides, or by somatic gene transfer. Overexpression of several genes in the heart has been shown recently to augment cardiac performance in laboratory animal models [182]. If the latter approach were taken, it has to be born in mind, however, that overexpression of a particular gene should be driven by a promoter which is specific for human ventricular tissue. In summary, detailed knowledge of the molecular mechanisms connected with the hypertrophy reaction and its transition into heart failure is a prerequisite for the development of new potential therapeutic strategies as well as of more adequate animal models to test them in vivo.

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